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(54) Title: **CHITOBIASE AS A REPORTER ENZYME**

(57) Abstract: The present invention relates to reporter gene constructs encoding a cytoplasmic form of chitobiase (N,N'-diacetyl-chitobiase) and methods of using these reporter gene constructs. The use of a cytoplasmic form of chitobiase as a reporter enzyme is generally applicable to the study of gene expression in organisms which do not contain N-acetyl- β -D-glucosaminidases.

WHAT IS CLAIMED IS:

1. A method for characterizing a promoter comprising:
providing a construct comprising said promoter operably linked to a nucleic acid encoding a
cytoplasmic form of chitobiase;
5 introducing the construct into host cells; and
identifying sequences in said promoter which regulate transcription levels.
2. The method of Claim 1, wherein said cytoplasmic form of chitobiase lacks a signal sequence.
3. The method of Claim 2, wherein said nucleic acid encoding a cytoplasmic form of chitobiase encodes a
fusion protein, said fusion protein comprising a cytoplasmic form of chitobiase fused to a heterologous polypeptide.
- 10 4. The method of Claim 1, wherein said nucleic acid encoding a cytoplasmic form encodes a cytoplasmic
form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. O-7*, *Arabidopsis*
thaliana, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium*
discoideum, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*,
Mus musculus, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia*
15 *marcescens*, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio*
furnissii, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.
5. The method of Claim 1, wherein said method of identifying sequences which are involved in directing
transcription comprises mutagenizing said promoter.
6. The method of Claim 1, wherein said method of identifying sequences which are involved in
20 transcription comprises constructing deletions in said promoter.
7. A method for identifying a regulatory element capable of directing or regulating transcription within a
test nucleic acid sequence comprising:
providing a construct comprising said test nucleic acid sequence operably linked to a nucleic acid
encoding a cytoplasmic form of chitobiase;
25 introducing said construct into host cells; and
determining the level of chitobiase activity.
8. The method of Claim 7, wherein said cytoplasmic form of chitobiase lacks a signal sequence.
9. The method of Claim 8, wherein said nucleic acid encoding a cytoplasmic form of chitobiase encodes a
fusion protein, said fusion protein comprising a cytoplasmic form of chitobiase fused to a heterologous polypeptide.
- 30 10. The method of Claim 7, wherein said nucleic acid encoding a cytoplasmic form encodes a cytoplasmic
form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. O-7*, *Arabidopsis*
thaliana, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium*
discoideum, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*,
Mus musculus, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia*

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marcescens, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio furnissii*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.

11. The method of Claim 7, wherein said reporter gene construct is introduced transiently.
12. The method of Claim 7, wherein said reporter gene construct is introduced stably.
- 5 13. The method of Claim 7, wherein said host cells are selected from the group consisting of prokaryotic cells and eukaryotic cells.
14. The method of Claim 7, further comprising permeabilizing or lysing said host cells.
15. The method of Claim 14, wherein said permeabilizing or lysing step comprises treating said host cells with toluene.
- 10 16. The method of Claim 7, wherein said step of determining the level of chitinase activity is selected from the group consisting of measuring the amount of a chemiluminescent product produced from a substrate, measuring the amount of a fluorescent product produced from a substrate, measuring the amount of light absorbed by a product produced from a substrate and measuring a decrease in the amount of a detectable substrate.
17. The method of Claim 7, wherein said step of determining the level of chitinase activity comprises determining the level of *p*-nitrophenol released from a substrate.
- 15 18. The method of Claim 7, wherein said test nucleic acid sequence comprises a portion of genomic DNA.
19. The method of Claim 7, wherein said step of determining the level of chitinase activity comprises determining the level of chitinase activity after exposing said host cells to a desired set of environmental conditions.
20. The method of Claim 7, wherein said step of determining the level of chitinase activity comprises determining the level of chitinase activity after contacting said host cells with a compound to be tested for its influence on the level of transcription from said regulatory element.
- 20 21. A method of detecting successful transformation, comprising the steps of:
 - introducing a nucleic acid encoding a cytoplasmic form of chitinase into host cells; and
 - detecting chitinase expression in said host cells.
- 25 22. A fusion protein-reporter gene construct comprising a promoter operably linked to a nucleic acid encoding a cytoplasmic form of chitinase fused in frame with a nucleic acid encoding a heterologous polypeptide, wherein said heterologous polypeptide is not -galactosidase or a portion thereof and wherein said heterologous polypeptide does not contain a signal peptide.
23. The nucleic acid of Claim 22, wherein said nucleic acid encodes a cytoplasmic form of chitinase
 - 30 obtained from an organism selected from the group consisting of *Alteromonas* sp. O-7, *Arabidopsis thaliana*, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium discoideum*, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*, *Mus musculus*, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas* sp. S9, *Rattus norvegicus*, *Serratia marcescens*, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio furnissii*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.
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24. The nucleic acid of Claim 22, further comprising a λ site-specific recombination sequence.
25. A reporter gene construct comprising plasmid pJMF3.
26. A reporter gene construct comprising plasmid pJMF4.
27. A reporter gene construct comprising plasmid pDYK9.
- 5 28. A reporter gene construct comprising plasmid pDYK11.
29. A host cell comprising the construct of Claim 22.
30. The host cell of Claim 29 wherein said nucleic acid is integrated into a chromosome of said cell.
31. The host cell of Claim 29, wherein said nucleic acid is transiently expressed in said host cell.
32. A nucleic acid encoding a cytoplasmic form of chitobiase in which the signal sequence of native
10 chitobiase has been inactivated or deleted.
33. The nucleic acid of Claim 32, wherein the signal sequence has been mutated to inactivate it.
34. An isolated or purified polypeptide comprising a cytoplasmic form of chitobiase fused in frame with a
heterologous polypeptide, wherein said heterologous polypeptide is not -galactosidase or a portion thereof and
wherein said heterologous polypeptide does not contain a signal peptide.
- 15 35. An isolated or purified polypeptide comprising a cytoplasmic form of chitobiase in which the signal
peptide of native chitobiase has been inactivated or deleted.
36. The polypeptide of Claim 35, wherein the signal sequence has been mutated to inactivate it.
37. A method for monitoring the activity of a promoter comprising:
providing a construct comprising said promoter operably linked to a nucleic acid encoding a
20 cytoplasmic form of chitobiase;
introducing said construct into host cells; and
determining the level of chitobiase activity.
38. The method of Claim 37, wherein said cytoplasmic form of chitobiase lacks a signal sequence.
39. The method of Claim 38, wherein said nucleic acid encoding a cytoplasmic form of chitobiase encodes a
25 fusion protein, said fusion protein comprising a cytoplasmic form of chitobiase fused to a heterologous polypeptide.
40. The method of Claim 37, wherein said nucleic acid encoding a cytoplasmic form encodes a cytoplasmic
form of chitobiase obtained from an organism selected from the group consisting of *Alteromonas sp. O-7*, *Arabidopsis*
thaliana, *Bacillus subtilis*, *Bombyx mori*, *Bos taurus*, *Caenorhabditis elegans*, *Candida albicans*, *Dictyostelium*
discoideum, *Entamoeba histolytica*, *Felis catus*, *Homo sapiens*, *Korat cats*, *Lactobacillus casei*, *Leishmania donovani*,
30 *Mus musculus*, *Pisum sativum*, *Porphyromonas gingivalis*, *Pseudoalteromonas sp. S9*, *Rattus norvegicus*, *Serratia*
marcescens, *Streptomyces plicatus*, *Streptomyces thermoviolaceus*, *Sus scrofa*, *Trichoderma harzianum*, *Vibrio*
furnissii, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.
41. The method of Claim 37, wherein said reporter gene construct is introduced transiently.
42. The method of Claim 37, wherein said reporter gene construct is introduced stably.

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43. The method of Claim 37, wherein said host cells are selected from the group consisting of prokaryotic cells and eukaryotic cells.

44. The method of Claim 37, further comprising permeabilizing or lysing said host cells.

5 45. The method of Claim 44, wherein said permeabilizing or lysing step comprises treating said host cells with toluene.

46. The method of Claim 37, wherein the step of determining the level of chitobiase activity is selected from the group consisting of measuring the amount of a chemiluminescent product produced from a substrate, measuring the amount of a fluorescent product produced from a substrate, measuring the amount of light absorbed by a product produced from a substrate and measuring a decrease in the amount of a detectable substrate.

10 47. The method of Claim 37, wherein said step of determining the level of chitobiase activity comprises determining the level of *p*-nitrophenol released from a substrate.

48. The method of Claim 37, wherein said step of determining the level of chitobiase activity comprises determining the level of chitobiase activity after exposing said host cells to a desired set of environmental conditions.

15 49. The method of Claim 37, wherein said step of determining the level of chitobiase activity comprises determining the level of chitobiase activity after contacting said host cells with a compound to be tested for its influence on the level of transcription from said regulatory element.

50. The method of Claim 49, wherein said compound comprises a compound to be tested for activity as a drug.